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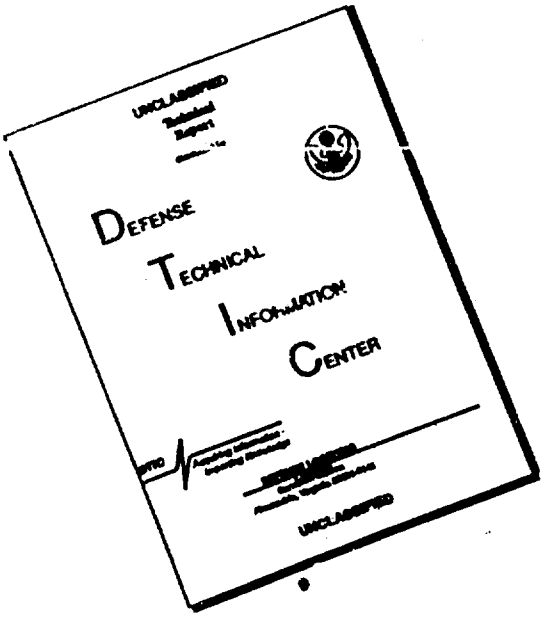

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SYNCHRONOUS PROPAGATION OF MICROORGANISMS AS A METHOD OF STUDYING THEIR BIOLOGY (REVIEW OF THE LITERATURE)

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Until recently the patterns of growth and development of microorganisms were studied by investigators using populations in the billions in which the physiological state of the individual cells was highly varied. Thus, conclusionswere based on averaged results that did not reflect the properties of the individual microbial cell at different times in its life. The effort to obtain populations of microorganisms all of whose cells might be in the same growth stage led investigators to synchronize their reproduction. The method involves, in essence, using agents of one kind or another to bring the microbial population into the same physiological state, one criterion of which is the simultaneous (synchronous) division of almost all the cells.

The first attempts at synchronization date back to 1923 when Sherman and Cameron (cited by Jacherts) noted a doubling of the number of cells in populations of E. coli after exposure to low temperatures (cold shock). Hershey used fractional sedimentation to separate shigella cultures into cells of different sizes. Their subsequent growth was marked by the rhythmic division of small and large cells. The synchronization method gained widespread popularity after the work of Tamiya et al. and Barner and Cohen who regarded synchronized populations as a single organism with characteristic physiological properties for each stage in its development.

During the past 10 years synchronized reproduction has been observed in microbes, protozoans, algae, tissue culture cells. The

microorganisms studied included the intestinal bacteria, mouse typhus salmonellas, pneumococci, and diphtheria bacteria.

The methods of synchronous propagation are numerous and varied. They can be divided into three large groups according to the nature of the action: mechanical selection, physical, and chemical-biological.

The methods based on mechanical selection of asynchronously dividing populations with some similar characteristics are called natural or selective. Some of them are widely used in microbiological research. For example, Maruyama and Yanagita, Lark, Abbo and Pardee, Anderson and Pettijohn, and others obtained comparable results by differential centrifugation of E. coli, Saccharomyces cerevisiae, and Chlorella. Prescott and Golub observed the effect of synchronized propagation of Amoeba proteus and Saccharomyces in the initial stages of development from a single cell (micromanipulation). Spektorov and Lin'kova suggested the simple method of natural sedimentation to synchronize Chlorella propagation. We may also include here the studies of Young and Fitz-James who achieved synchronous propagation of Bacillus cereus through development of the population from selected spores.

Thus, the first group of methods involves the mechanical selection of cells from a population according to the volume, weight, or certain forms of existence (in the case of selection of spores). The use of selective methods is based on the assumption that such properties as cell volume and weight reflect a particular physiological state. However, these methods are not suitable for all kinds of microorganisms. For example, selection involving centrifugation or filtration (Hershey, Jacherts) is unsuitable for the mobile forms of bacteria which have well-developed flagella.

The second group of methods is based on the use of physical agents, the commonest being temperature (Lark and Maale, Hunter-Szybalska et al., Biryuzova and Nikitina, Hess and Shon, and others). Synchronization of the populations is achieved by a single (shock) or repeated (changes in temperature between two levels) exposure to higher or lower temperatures than those known to be optimal for the particular species. The temperature effect blocks the process of division (Swann) and slows but does not halt cell growth and development (Scherbaum), resulting ultimately in a uniform population. Then after the shock is neutralized, 70 to 95% of the cells start to divide simultaneously. This type of action is used largely for microbes, protozoans, and tissue culture cells. Another technique is to expose microorganisms to sublethal doses of X-rays. Spoerl and Looney induced simultaneous budding in a Saccharomyces cerevisiae culture

by exposing it to 1.85 kr/hour of X-rays. Still another type of physical action is the periodic alternation of light and darkness, a method described in the survey of Goryunova et al.

The third group of factors alters the metabolism of the culture under study. For example, Scott, Xeros, and Lark and Lark after treating cultures of microorganisms and monolayer tissue cultures (Chang cells) with deoxyribosides, ribonucleosides, and 2,4-dinitrophenol observed simultaneous division, which they attributed to the suppression of DNA synthesis by the above substances. This view is consistent with the findings of Langer and Klenow who showed that deoxyadenosine retards DNA synthesis in Ehrlich's ascites tumor cells. One of the most popular techniques is the so-called metabolic shock. Barner and Cohen, Burns, Stevenson, and others left populations of the thymine-dependent mutant of E. coli, deoxyriboside mutant of Lactobacillus acidophilus, or arthrobacteria (which require vitamin B_{12} for their existence) without the corresponding dependency factor. The subsequent addition of thymine, deoxyriboside, or vitamin B_{12} to the medium caused these microorganisms to reproduce simultaneously.

Synchronization can be achieved by using "hungry" media from which the most important constituents have been eliminated or decreased (Campbell, Greve, and others). The subsequent addition of the deficient substance or transfer of the cells to a rich medium stimulates synchronous division.

Despite the variety of methods available, it is not always possible to achieve the desired degree of synchronization. In such cases agents are combined. For example, Perry combined temperature and metabolic shock to induce simultaneous reproduction in E. coli. Williamson and Scopes, in addition to temperature shock and cultivation in a hungry medium, subjected a Saccharomyces cerevisiae culture to centrifugation and treatment with the intestinal juice of snails to remove paired and unpaired buds.

There are several hypotheses to explain the mechanism of cell division in synchronized cultures. Hotchkiss thinks that temperature shock suppresses some enzymatic processes related to cell division, while the microbial mass is provided with all that it needs for growth and development. Removal of the blocking factor promotes the synthesis of the compound responsible for cell multiplication. If present in adequate quantities, almost all the cells in the population start to divide at the same time. Williamson and Scopes assume the existence of several "trigger" mechanisms. The substances responsible for the budding of yeast can be synthesized regardless of the growth of the mass. However, the authors do not rule out the

possible influence of structural reorganization in the membrane, although they do not explain the mechanism of this process. Some confirmation is to be found in the data of Nickerson and Falcone who studied the activity of protein disulfide reductase in a mutant of Candida albicans. They concluded that division in yeasts starts with the reduction of the disulfide bonds of the chemical structures in the membrane. Scherblaum tried to identify the multiplication factor with a special fraction of DNA and a thymine-containing coenzyme. He bases his view on the close relationship existing between DNA synthesis and cell division in experiments with a single cold shock, which halts cell division but permits the synthesis of RNA and proteins to continue. The author's hypothesis of a thymine-containing coenzyme may be linked to the observations of Barner and Cohen and those of Prusoff on the possibility of achieving synchronization by adding thymine to a medium containing thymine-dependent mutants. However, synchronization may be achieved with other dependent mutants by adding the corresponding factor to the medium.

The main purpose of the research on models of simultaneously divising cultures was to search for the factors responsible for cell growth and multiplication. Considerable attention was focused on the dynamics of the synthesis of DNA, protein RNA, and phosphorus compounds. The great majority of experiments performed on different objects using various methods of synchronization showed that DNA is synthesized unevenly in the course of a single generation (Ogur et al., Iwamura, Nygaard et al., Zaytseva, and others). For example, the amount of DNA doubles immediately after simultaneous division if the cells divide in two. In the sporulation phase of Chlorella, on the other hand, DNA increases in proportion to the number of newly formed cells. These findings are in agreement with present-day thinking on the constant DNA content of each nucleus. Young and Fitz-James, Schaechter et al., Barner and Cohen, and Abbo and Pardee reached a different conclusion. They showed that DNA synthesis in synchronized Bacillus cereus and E. coli cultures takes place continuously. These results may be due to the nature of the techniques used in the work (synchronized B. cereus cultures were obtained from selected spores).

Studies on the dynamics of RNA synthesis without separation into individual fractions showed that the substance is synthesized continuously during an entire generation (Nygaard et al., Abbo and Pardee). But separate analysis of the sedimentary and supernatant fractions of RNA revealed some differences in the nature of the synthesis of these compounds (Maruyama and Lark). RNA from the sedimentary fraction was found to be synthesized periodically, whereas the fraction obtained from the supernatant is synthesized continuously. Periodic synthesis of RNA was observed in yeasts as well as in

bacteria. A yeast culture was obtained from a single cell (Mitchison and Walker). In 1963 Zaytseva studied separately different kinds of RNA in the course of the synchronous development of Azotobacter, concluding that the synthesis of soluble and information RNA is distinctly periodic. The maximum accumulation occurs before division, the minimum afterward, but the amount of ribosomal RNA increases almost exponentially with increase in protein.

Study of the dynamics of protein synthesis in models of simultaneously dividing Azotobacter cultures showed that protein is synthesized continuously throughout an entire generation (Zaytseva et al.). However, in studying separate protein fractions she found that the synthesis of polynucleotide phosphorylase, RNA polymerase, and nucleoside phosphokinase comes to a virtual halt during the period of division. The author believes that the cyclical nature of the process is masked by the continuous proteosynthesis of most protein fractions if the total protein content is analyzed without fractionating it.

The dynamics of the metabolism of the so-called acid-soluble nucleotide reserve has a direct bearing on DNA and RNA synthesis, for such nucleotides are regarded as precursors in the synthesis of the nucleic acids. The studies of Zaytseva et al. and of Maruyama and Lark on models of synchronized Azotobacter cultures showed that the amount of acid-soluble mononucleotides increases greatly before each cell division and decreases sharply thereafter. The authors conclude that changes in the size of the reserve may affect the synthesis of the nucleic acids. The free nucleotides of the metabolic reserve seem to function as regulators of their biosynthesis.

The elements of metabolism connected with the energy balance of the cell are particularly important. Adenosinetriphosphate and polyphosphates are directly involved in these processes. Zaytseva and Plezner on synchronized Azotobacter vinelandii and Tetrahymena pyriformis cultures showed that quantitative changes in the adenosinetriphosphate expended in the course of cell division have the same rhythm as oxidative phosphorylation, while the polyphosphates undergo substantial quantitative changes in the period of cell division of synchronized populations (Zaytseva et al., Soll et al.). Meanwhile the amount of acid-insoluble polyphosphates sharply decreases with simultaneous increase in the acid-soluble polyphosphates, especially mineral phosphorus. The authors believe that high-molecular acid-insoluble polyphosphates degrade to low-molecular fragments, orthophosphate, liberating the phosphorus and energy that are needed for cell division.

In studying the mechanism of division, the investigators focused on enzymes and coenzymes. The research of Sylven et al. on

the dynamics of some enzyme systems in synchronized yeast populations showed that the activity of the proteinases, especially the dipeptidases, increased as soon as division started. The cycles of intensified proteolysis were also linked to division. The investigators think that these enzymes take part in intercellular reactions that lead to replenishment of the mixture of metabolic amino acids needed for protein synthesis. As for the coenzymes of the enzyme systems involved in synthesis of the polysaccharides in the cell walls which, according to Kotel'nikova, are uridindiphospho derivatives of sugars, it has been found that they accumulate rapidly in the interval between the first and second simultaneous divisions (Zaytseva et al.). This indicates that in preparing to divide, the cell provides itself with coenzymes of the enzyme systems and precursors participating in the synthesis of various polysaccharides and phosphorylated sugars.

A few studies on models of simultaneously dividing cultures dealt with other aspects of the biology of the microbial cell, e.g., lysogenicity of cultures in relation to the phase of cell growth. According to Lark and Maale, the frequency of lysogenicity of S. typhimurium suddenly doubles 6 minutes before division, or the time required for the amount of DNA to double. Romig et al. subjected a synchronized E. coli culture to lethal doses of radiation at different times during the cell's life cycle. They found that young cells died in larger numbers just after dividing than they did before. Among these cells they discovered many mutants. The increase in number of mutations and frequency of lysogenicity of the cells before dividing seem to depend not only on the process of DNA reduplication but also on the increased DNA content of the cell and, consequently, increased probability of mutations arising.

All the factors mentioned above convincingly show that the method of synchronous propagation of microorganisms provides broad opportunities for studying their physiology. As is evident from the research described, certain metabolic processes in the cell are closely bound up with its division. It has been found that prior to the start of cell division there is an increase in the amount of DNA and individual RNA fractions (S and m) and greater activity of the enzyme systems (RNA polymerases, polynucleotide phosphorylases, nucleoside phosphokinases, proteinases, and dipeptidases). Zaytseva (1963) believes that the similarity in dynamics of accumulation of the individual constituents may be indicative of a certain degree of coordinated action in protein synthesis and cyclical nature of cell division. However, the factors involved and the mechanism of synchronous propagation are still obscure. Further research may throw light both on the mechanism of synchronous propagation and on the propagation of microorganisms in general and thus open up tempting prospects for controlling the processes of their growth and multiplication.

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